two and two, by twenty-one lines, then any seven planes that contain these twenty-one lines will osculate a second cubic curve.

EDWIN BIDWELL WILSON

SPECIAL ARTICLES

PRELIMINARY STUDIES ON INTRACELLULAR DIGESTION AND ASSIMILATION IN AMPHIBIAN EMBRYOS [1]

BY means of a double stain of janus green and neutral red in an isotonic salt solution, the initial dilution of each stain being about 1:10,000, the yolk globules in the living cells of *Amblystoma* embryos may be differentiated into two types, which, for convenience of description, I designate as "alpha" and "beta" globules. The alpha globules stain selectively with janus green, at first greenish blue and then pinkish, presumably upon reduction of the dye. The beta globules stain selectively with the neutral red, and are by far the more numerous in the cell. When the same dyes are used singly in a dilution of 1:30,000 the alpha globules are relatively inert towards the red, and the beta globules are not stained by the green. In smears of living embryos which have been fixed upon the cover glass with the acetic-osmic bichromate mixture and stained with acid fuchsin according to the method of Bensley,[2] for mitochondria, the beta globules stain a deep, brilliant red while the alpha globules take on a duller tint, bordering on purple. The two types of globules may be similarly differentiated in sections prepared according to this method.

In smears of living cells which have been stained in janus green, alpha globules may be found here and there with deeply stained, blue excrescences upon their surface. These structures may be described as "alpha bodies." These are particularly distinct after the globule on which they occur has begun to take on the pinkish tint. They frequently appear as rows of slightly elongated masses connected by slender threads of the same kind of substance. In optical section some of them seem to dip into the substance of the globule while others project in varying degree above it. Some even have a very slight attachment to the globule. In other instances similarly staining substance is arranged in relatively coarse bands with ragged outline, a condition to which I shall refer again in considering the toxic action of the dye.

The different forms of alpha bodies I regard as indicative of different stages in their development. I have seen them in numerous cases arranged in rows over the surface of the globule as separate and distinct bodies. In this condition they have the form and color of mitochondria in the same preparation. In one instance, in fact, after I had begun to draw a globule with these separate and distinct alpha globules on its surface, I observed that some of the alpha bodies were changing their position relative to each other, and, giving continuous and close observation to those bodies, I saw some of them break loose from the globule and become indistinguishable in form and color from mitochondria which appeared elsewhere in the same preparation. Alpha bodies are visible also in smears and sections made according to Bensley's method for mitochondria as noted above.

Similarly there appear on some beta globules structures which may be called "beta bodies." These stain a deep red in contrast with the more delicately tinted body of the globule. In some respects they resemble in general structure the alpha bodies, but they are of a coarser nature. In some instances there is a hull of this substance around the greater part of the globule. Upon other globules it appears in ridges or as a chain of angular bodies. In smears of living cells I have seen beta bodies,

[1] When this paper was written I was not acquainted with the contribution of C. Saint-Hilaire: "Ueber die Veränderungen der Dotterkörner der Amphibien bei der intracellulären Verdauung," *Zoologische Jahrbücher, Abt. f. Allg. Zool. u. Physiol.*, B. 34, Heft 2. After a careful study of his results I am convinced that Saint-Hilaire has not seen my "alpha bodies." Otherwise, my observations, in many respects, are in striking agreement with his. The differences in matters of interpretation can not be discussed here.

[2] Bensley, R. R., "Studies on the Pancreas of the Guinea Pig," *American Journal of Anatomy*, Vol. 12, No. 3.

also, break loose from the globule. In the free condition they become indistinguishable from the free bodies which are abundant in the cells of amphibian embryos and which are ordinarily regarded as pigment. These pigment granules, although having a color of their own, at least upon their surface, stain deeply with neutral red. The beta bodies give the reaction for fat with Herxheimer's method.

That the beta bodies can not be degeneration products in the strict sense is evidenced by the fact that yolk globules remain intact for a long period in dishes of putrefying embryos, and that, in this condition, they do not stain selectively in neutral red and nothing like beta bodies can be found upon them. However, such globules, taken from disintegrating embryos, after they have been ingested by large protozoa, stain selectively in the food vacuoles of the living organism. In fact, large ciliates which have been feeding in dishes where embryos are disintegrating in a solution of neutral red, become gorged with deep red granules in dense masses. In one instance I have seen a swimming ciliate discharge a number of these granules, apparently as dejecta.

In the study of the reaction to janus green of yolk globules that have been ingested by protozoa I have met difficulties which have not been entirely overcome, but in one large ciliate I have succeeded in getting the reaction of two food vacuoles to the double stain of janus green and neutral red. In this case the surface of the globule stained a dense red and the other contents of the vacuole around the yolk globule a faint blue which changed in time to faint pink. The latter reaction was delicate but unmistakable.

A study of the artificial digestion, also, of yolk globules which have been taken from dead embryos supports the view that the selective staining of yolk globules and the bodies on their surface is due to processes of digestion. When such globules are digested in a mixture of pancreatin and neutral red many stain selectively and bodies appear on their surface which resemble beta bodies in living preparations. With prolonged digestion in pancreatin and neutral red the solution becomes yellow, and the core of the digesting globules yellow, while the bodies on their surface are deep red. Such reactions do not occur in digestion with pepsin in solution with neutral red, either with or without the addition of hydrochloric acid, although there is positive evidence of digestion in the mixture. Digestion with pepsin and janus green, however, brings about selective staining of globules which, during digestion, break up into very small bodies. These bodies stain a deep blue or blue-green. Such bodies occur, also, upon the surface of more faintly stained blue globules, in which case they resemble the alpha bodies of living preparations. Although they are usually larger than the typical alpha bodies, some of them are of about the same size.

In preparations of living cells stained with the double stain of neutral red and janus green I have on several occasions found an individual globule which had both alpha and beta bodies attached, the alpha bodies situated in bluish areas and the beta bodies in regions of fainter red. One such globule I had under observation for over eight and one half hours. During the latter part of this period beta bodies became detached from the globule while the globule became much reduced in size and retained the bluish tint over a relatively larger area than formerly. During this time an alpha body, also, disappeared from the surface of the globule, but it could not be recognized afterwards in the free condition as were the beta bodies. The latter, in the free condition, assumed the characteristics of the so-called pigment granules in the same preparation.

These preliminary observations have left a strong conviction in my mind that, in the digestion and assimilation of yolk in these embryos, enzymes effect a cleavage of the superficial substance of the globule; that, following this cleavage, the end-products of the process segregate into alpha bodies on the one hand and beta bodies on the other, and that the alpha bodies, probably undergoing some chemical change in the meantime, become free as mitochondria in the process of assimilation

into protoplasm, while the beta bodies are at this stage of development essentially a residue which later in cytomorphosis, possibly only after the circulatory system has assumed its nutritive rôle, may undergo further digestion.

This interpretation is further supported by the fact that janus green manifests much greater toxicity than does neutral red when embryos are grown in like dilutions of these dyes. This difference in toxic action becomes intelligible when one recognizes that it is the processes that are leading up to the construction of protoplasm that are obstructed by the reaction of janus green with the cell, whereas it is only the residue, so to speak, of these processes that is attacked by the neutral red. The latter dye has, however, a very considerable toxic action, the intracellular effects of which can be readily recognized. The yolk globules of embryos that have grown some time in a solution of neutral red have enormous, deeply stained red excrescences upon their surface. Many small structures like beta bodies in the fresh smears of living cells occur also under such conditions. The excessively large excrescences, which form large buds and separate into deeply staining, small globules, can not be regarded, of course, as perfectly normal. Neither are they degeneration products in the strict sense, for, as noted above, they do not occur on globules of degenerating tissues. They should be regarded, rather, as the result of normal processes that have been obstructed by the reaction of the products with the dye. That there is a more stable chemical compound established here is evidenced by the fact that these excrescences can be fixed with ammonium molybdate and preserved in microscopic sections, whereas neutral red stains of other structures in the cell can not be preserved by this method. Unusually large excrescences, also, which I have frequently seen on alpha globules, are probably the expression of the toxic action of janus green.

The experiments which have led me into this field began as a search for a method of detecting polarity in cells and physiological gradients within the embryo, my purpose being to correlate my work on the growth of the reflex arc in its relation to the development of behavior with recent researches upon gradients in lower organisms, particularly by Child.[3] In their bearing upon this original plan my results seem to justify the use of janus green and neutral red as indicators of digestion and assimilation of yolk in amphibian embryos. Beyond this, it seems to me, my observations give a clue, not only to the mechanism of intracellular digestion and assimilation of yolk, but also to the nature of the toxic action of the dyes that have been employed. My observations, however, are not presented here as conclusive evidence. They require critical review and extensive corroboration. But, awaiting the opportunity of another season, I feel justified in making this preliminary report, particularly in the hope of enlisting the interest of other biologists in the amphibian embryo as a unique source of information upon important phases of cellular biology. It would be interesting to know, for instance, the cytological side of the toxic action of the phenolic compounds which Gortner and Banta[4] used on amphibian embryos. With reference to mitochondria, my interpretation that they are derived in the amphibian embryo from yolk through the formation of structure which I call "alpha bodies" is wholly in accord with the conclusion of Cowdry[5] that mitochondria are associated with metabolism, and it is not at variance with the observations of M. R. and W. H. Lewis[6] that

[3] Child, C. M., "Studies on the Dynamic Morphogenesis and Inheritance in Experimental Reproduction, VIII., Dynamic Factors in Head-determination in *Planaria*," *The Journal of Experimental Zoology*, Vol. 17, No. 1.

[4] Gortner, R. A., and Banta, A. M., "Notes on the Toxicity of Dilute Solutions of Certain Phenolic Compounds, etc.," *Biochemical Bulletin*, Vol. 3, Nos. 11, 12.

[5] Cowdry, E. V., "The Comparative Distribution of Mitochondria in Spinal Ganglion Cells of Vertebrates," *The American Journal of Anatomy*, Vol. 17, No. 1.

[6] Lewis, M. R., and W. H., "Mitochondria (and Other Cytoplasmic Structures) in Tissue Cultures," *The American Journal of Anatomy*, Vol. 17, No. 3.

mitochondria in the cells of the chick embryo increase in size and divide by fission, when the cells are grown in vitro. If, as my observations indicate, mitochondria are involved in the anabolic phase of metabolism, one would expect them to grow in the cell of the chick embryo by accretion from end products of digestion absorbed by the cell; whereas in the amphibian embryo the food is stored within the cell as relatively stable substance and the whole transformation from food to protoplasm must take place in situ. So long as the cell is nourished from yolk which it contains, the mitochondria, I believe, grow upon the surface of the yolk globule. They may be certain end products of digestion, or they may be synthesized out of certain of the end products of digestion. However, before accepting this hypothesis it is important to know whether mitochondria occur in cells which have been deprived of their yolk by centrifuging. The work of Banta and Gortner,[7] and particularly that of Jenkinson,[8] upon the development of centrifuged amphibian eggs should be extended into the cytological field to determine wherein the mechanism is deficient in those cells which do not develop normally. Furthermore, the interpretations here offered, in so far as they relate to mitochondria, must be qualified by the consideration that their validity rests largely upon the nature of the bodies in the protoplasms which I have regarded as mitochondria. My judgment on this point is based upon the use of janus green as a vital stain and of Bensley's acetic-osmic-bichromate method, the two methods which, taken together, seem to be accepted as the nearest approximation to a specific test for mitochondria now at our command. But regardless of theoretical considerations, the observations which have been described are, I believe, substantially correct, and they are presented in this form with the hope of stimulating interest in a field of study which affords peculiar opportunity for making a definite advance in our knowledge of the mechanics of the cell, particularly in relation to the growth of the organism.

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[7] Banta, A. M., and Gortner, R. A., "Accessory Appendages and Other Abnormalities Produced in Amphibian Larvæ through the Action of Centrifugal Force," *The Journal of Experimental Zoology*, Vol. 18, No. 3.

[8] Jenkinson, J. W., "The Relation between the Structure and the Development of Centrifuged Eggs of the Frog," *Quarterly Journal of Microscopical Science*, April, 1914.

TRAINS OF BEATING LIGHT WAVES

If two spectra, having the same longitudinal axis but reversed in color (*i. e.*, respectively red-violet and violet-red), are brought to interfere, the interference should occur only along the single transverse line of coincidence and therefore be inappreciable. If it is visible, then light waves of slightly *different* wavelengths, lying symmetrically on either side of the common transverse axis, must also be capable of interference in optics, in complete analogy with the case of musical beats in acoustics. After long searching I found that the occurrence of the phenomenon in question can be shown experimentally. Its scintillating appearance is exceedingly striking. It is complete within a transverse strip of the spectrum but one half to one third the width of the sodium lines. It partakes of the general characters of elliptic interferences however, except that the ellipses are now extremely eccentric (needle-shaped in other words) and confined to a single color. If the given width be regarded as the distance between two fringes and estimated as $d\lambda = 2.4 \times 10^{-8}$ cm., if x be the distance along the axis of propagation within which one reenforcement occurs, then

$$x = \lambda^2/d\lambda = 36 \times 10^{-10}/2.4 \times 10^{-8} = .15 \text{ cm.},$$

or the limiting group wave-length of the light waves is over a millimeter. Details and allied results, for which there is no room here, will be found in the complete paper, now in the hands of *The American Journal of Science*.

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